Pulsed stimulated Brillouin microscopy

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Abstract: Stimulated Brillouin scattering is an emerging technique for probing the mechanical properties of biological samples. However, the nonlinear process requires high optical intensities to generate sufficient signal-to-noise ratio (SNR). Here, we show that the SNR of stimulated Brillouin scattering can exceed that of spontaneous Brillouin scattering with the same average power levels suitable for biological samples. We verify the theoretical prediction by developing a novel scheme using low duty cycle, nanosecond pulses for the pump and probe. A shot noise-limited SNR over 1000 was measured with a total average power of 10 mW for 2 ms or 50 mW for 200 µs integration on water samples. High-resolution maps of Brillouin frequency shift, linewidth, and gain amplitude from cells in vitro are obtained with a spectral acquisition time of 20 ms. Our results demonstrate the superior SNR of pulsed stimulated Brillouin over spontaneous Brillouin microscopy.

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1. Introduction

Brillouin light scattering microscopy is a non-contact and label-free technique for probing the mechanical properties of cells and tissues [1] with application in the study of tumor growth [2,3], cell hydromechanics [4,5], tissue repair [6], cornea biomechanics [7,8], biomaterials [9,10], etc. Conventional Brillouin microscopy measures thermodynamic fluctuations or naturally generated mechanical pressure waves in a sample, but this spontaneous process has an inherently weak scattering efficiency, which is independent of the probe power [11,12]. To overcome this limitation, nonlinear Brillouin scattering techniques have been exploited [13-15]. The mechanical waves are generated by dynamic electrostriction resulting from the interactions of pump and probe beams, and the Brillouin scattering efficiency increases with the optical intensity of the pump beam [16]. The recent demonstration of stimulated Brillouin scattering (SBS) [17] using continuous-wave (cw) pump and probe beams required a substantially high power (~265 mW) to obtain a signal to noise (SNR) comparable to the SNR of state-of-the-art spontaneous Brillouin microscopy. This power exposure level, however, is not desirable for low phototoxicity on biological samples such as live cells [18,19]. Here we demonstrate a pulse scheme for stimulated Brillouin microscopy. Our scheme uses nanosecond pulses for both pump and probe beams with high peak powers, but a modest level of average powers, to provide better SNR performance than the previous cw stimulated Brillouin microscopy even with significantly less power exposure on samples. We provide both theoretical and experimental frameworks of pulse-enabled SNR enhancement in Brillouin microscopy.

As the third-order nonlinear effect, SBS has long been investigated in many areas in physical sciences and optical engineering. The gain bandwidth of SBS in most solid materials and liquids is several tens to hundreds of MHz. Therefore, the SBS generation is typically done using nanosecond pulses. In fiber-optic telecommunications using pulsed modulation, significant SBS may build up in the long transmission line. Nanosecond pulses are commonly utilized in SBS for fiber-optic distributed sensing [20,21], optical signal processing [22], storage [23], and

delay [24], and on-chip Brillouin lasers [25–27]. While these applications take advantage of waveguide-assisted long acousto-optic interaction lengths typically in the order of centimeters to meters, the application of SBS to microscopy brings a somewhat unique challenge in that the effective acousto-optic interaction length is quite short in a range of 1-10 μ m, by tight focusing of optical beams. With the constraint in optical power to samples, the amplitude of SBS gain can be substantially low, and therefore, a detailed analysis of SNR, particularly in comparison to the linear spontaneous Brillouin process, will be useful.

1.1. Theoretical SNR comparison of spontaneous vs. stimulated Brillouin microscopy

The spontaneous Brillouin scattering cross section per solid angle is known to be $\sigma = \frac{\pi^2 V}{\lambda^4} \left(\frac{\gamma^2}{M}\right) k_B T \frac{(1+\cos^2\theta)}{2}$ [11,12], where λ is optical wavelength, M is longitudinal modulus, k_B the Boltzmann constant, T temperature, V volume of the interaction region, θ the scattering angle, and $\gamma = \rho \partial \varepsilon / \partial \rho$ the electrostriction constant where ε is dielectric constant and ρ is density [11]. The angle dependence reflects polarization dependence. $V = A\Delta z$, where A is the beam area and Δz is the effective acousto-optic interaction length. In confocal Brillouin microscopy, Δz is approximately 2 times the Rayleigh length $z_R = \frac{n\lambda}{\pi NA^2}$, where NA is the numerical aperture of the focused beam in the sample with a refractive index n. The scattering efficiency is $S = \sigma/A$. By integrating over the solid angle of confocal light collection, the theoretical maximum collection efficiency of Brillouin scattering in the confocal configuration is given by [28]

$$a \approx \frac{2\pi^2}{n\lambda^3} \left(\frac{\gamma^2}{M}\right) k_B T \tag{1}$$

In an ideal (lossless and low noise) system, we get $a \approx 5.4 \times 10^{-11}$ for $\lambda = 780$ nm from water at room temperature. The total optical power of Brillouin scattered light is equal to aP_0 , where P_0 is the optical power of the probe beam in the sample. The theoretical maximum SNR, defined as the total measured *power* ratio of signal to noise, is:

$$SNR_{1} = \frac{\left(\int_{0}^{T} \eta a P_{0} dt\right)^{2}}{\int_{0}^{T} \eta a P_{0} dt} = a\eta \left\langle P_{0} \right\rangle T$$
⁽²⁾

where η is the photon-to-electron conversion efficiency of photodetection ($\eta = 0.63$ at $\lambda = 780$ nm with 100% quantum efficiency), $\langle P_0 \rangle$ is the time-average optical power, and *T* is the integration time. $\frac{\eta}{h\nu} \langle P_0 \rangle T = N_{tot}$ is the total number of photoelectrons measured during the integration time.

Now consider the stimulated Brillouin scattering between pump and probe beams that are counterpropagating and overlap perfectly in a sample. The Brillouin gain for the probe light is expressed in $\frac{dP_r}{dz} = gI_pP_r$, where P_r denotes the probe power, I_p is the pump beam intensity, and $g(f) = \frac{\pi^2}{n^2\lambda^2} \frac{v}{c} \left(\frac{\gamma^2}{M}\right) \frac{2}{\pi\Gamma} \frac{(\Gamma/2)^2}{(f-f_B)^2 + (\Gamma/2)^2}$ is the Brillouin gain factor [16], where Γ is the Brillouin frequency shift, and $v = \sqrt{M/\rho}$ is the longitudinal wave velocity. For water ($\Gamma \approx 350$ MHz), we get $g(f_B) = 3.5 \times 10^{-5}$ µm/W at $\lambda = 780$ nm. The Brillouin gain for the probe is given by $G = gI_p\Delta z$, where Δz is the acousto-optic interaction length. Due to the dependence on the pump intensity, the gain decreases away from the focus, and Δz is in the order of the Rayleigh length. The focused Gaussian beam has a beam waist area $A = \frac{1}{2\pi} \frac{\lambda^2}{NA^2}$. Using $I_p = P_p/A$, where P_p is the pump power, we find $G(f_B) = bP_p$, with $b \approx 1.0 \times 10^{-4}$ W⁻¹ for water. In an ideal system, the probe power after the sample becomes $(1 + G)P_r$. For cw pump and probe beams, the shot-noise-limited SNR is

$$SNR_2(f_B) = \frac{\left(\int_0^T \eta GP_r dt\right)^2}{\eta \left(1+G\right) P_r T} \approx G^2 \eta \left\langle P_r \right\rangle T = b^2 P_p^2 \eta \left\langle P_r \right\rangle T \tag{3}$$

In non-ideal experimental systems with non-negligible electrical and other optical noises, in order to measure the weak signal on top of the background probe light it is necessary to modulate the pump peak power and extract the modulated amplitude of the probe. For instance, for cw cases the pump power is modulated sinusoidally for lock-in detection. In this case, $\langle P_p \rangle = 0.5P_p$. For pulsed cases, the pump is a train of pulses with a duty cycle (the ratio of the pulse width to the pulse period) of κ . Then, we get $\langle P_p \rangle = \langle P_p \rangle \kappa$. To measure the difference between two adjacent probe pulses with and without the pump, the duty cycle of the probe may be set to 2κ , and $\langle P_r \rangle = 2P_r\kappa$. This interlaced pulsed scheme is illustrated in Fig. 1. For the same $\langle P_p \rangle$ and pump duty cycle, this process reduces the SNR in Eq. (3) by 4 folds since only a half of the probe light interacts with the pump. If dual balanced detection is used to reduce the relative intensity noise (RIN) in the probe beam, SNR would be further reduced by 2-fold since shot noise is doubled in the dual balancing. With these factors considered, the SNR estimate in Eq. (3) is decreased by a factor of 8.



Fig. 1. Schematic of the interlaced pulse scheme for stimulated Brillouin microscopy. (a) An optical setup with a probe beam and a counterpropagating pump beam. (b) The timing diagram of pump pulses with a duty cycle κ and probe pulses with twice the repetition rate. The detector signal of the probe beam going through the sample is digitized and integrated with specified boxcar windows. The difference of the integrated values between each adjacent pair of windows (n0 without pump and n1 with pump) is recorded and plotted as the frequency difference between the pump and probe light is scanned. This produces a Brillouin spectrum.

To compare the spontaneous and stimulated regimes, we consider the same total optical power $\langle P_{tot} \rangle = \langle P_r \rangle + \langle P_p \rangle$ and $\langle P_{tot} \rangle = \langle P_0 \rangle$, where the bracket indicates time average. With the constraint on total power, SNR_2 is maximized when $\langle P_p \rangle = 2 \langle P_r \rangle$ and, thus, $P_0 = 3 \langle P_r \rangle$. Considering the factor of 8 reduction described above, we get

$$SNR_{3}(f_{B}) = \frac{b^{2} \langle P_{p} \rangle^{2}}{8\kappa^{2}} \eta \langle P_{r} \rangle T \xrightarrow{\langle P_{p} \rangle : \langle P_{r} \rangle = 2:1} \frac{b^{2} \langle P_{tot} \rangle^{2}}{54\kappa^{2}} \eta \langle P_{tot} \rangle T$$
(4)

For a given duty cycle κ , the SNR is proportional to $\langle P_{tot} \rangle^3$. For the same average power, the SNR increases with $1/\kappa^2$. This is a major advantage of the pulsed scheme.

Figure 2 depicts the theoretical SNR per 1 millisecond integration time as a function of the total average power on sample. The SNR of cw stimulated Brillouin microscopy (κ =0.5) is lower than that of spontaneous Brillouin microscopy even at $\langle P_{tot} \rangle = 200$ mW. With a duty cycle of 0.05, pulsed stimulated Brillouin microscopy has a higher SNR than spontaneous Brillouin microscopy for $\langle P_{tot} \rangle > 27$ mW. The breakeven point decreases to 2.7 mW with a duty cycle of 0.005.



Fig. 2. Theoretical plot of *SNR* per 1 millisecond integration time for different cases based on Eq. (2) for spontaneous Brillouin microscopy and Eq. (4) for stimulated Brillouin microscopy. The pulsed scheme with low duty cycle allows for a higher SNR than spontaneous Brillouin microscopy with low power exposure to samples.

In the above, we have compared the peak SNR's. Brillouin microscopy and spectroscopy involves measuring the peak of the Brillouin spectrum or the Brillouin frequency shift, f_B . In spontaneous scattering, the signal in Eq. (1) contains all frequency components, from which f_B is determined using a spectrometer. However, in stimulated Brillouin scattering, only one frequency difference is interrogated at a time. To determine f_B , therefore, the frequency difference f must be varied. Let us consider a linear tuning case where f is scanned over a range from $f_B - m\Gamma/2$ to $f_B + m\Gamma/2$, with a frequency step size of δf . So, a total of $N_s = m\Gamma/\delta f$ spectral points are acquired over a total duration of N_sT . We consider a general approximation for the uncertainty in the peak Brillouin shift: $\sigma_f \approx B\sqrt{\frac{\Gamma \ \delta f}{SNR(f_B)}}$, where B is a constant depending on the gain profile, fitting function, and noise type, as well as the frequency scan range (Supplemental document). We performed a computer simulation to find B = 0.8 for m > 1.5 and $B \approx (2m)^{-1.6} + 0.64$ for m < 1.5 (Supplement 1, Fig. S1). Previously, B = 0.89 was derived for large m using Gaussian fitting [29], and B = 0.87 for m = 1 were derived for a quadratic fitting method [30]. Inserting $\delta f = \frac{m\Gamma}{N_s}$, we can write

$$\sigma_f = B \sqrt{\frac{\Gamma \ \delta f}{SNR_3(f_B)}} = B(m) \sqrt{m} \frac{\Gamma}{\sqrt{SNR_3(f_B)N_s}}$$
(5)

In spontaneous Brillouin spectroscopy, the scattering photons are accumulated for the same integration time N_sT . Assuming there is no other noise background than the shot noise of the signal photons [31,32], computer simulation gives $\sigma_f \approx 0.8 \frac{\Gamma}{\sqrt{SNR_1N_s}}$. Therefore, the same uncertainty is obtained when $SNR_3(f_B) = m SNR_1$ for m > 1.5. A scan with m = 4, for instance, gives 6 dB penalty for stimulated Brillouin spectroscopy. When m < 1.5, the same uncertainty is obtained when $SNR_3(f_B) = (0.41m^{-1.1} + 0.8m^{0.5})^2 SNR_1$ for m < 1.5. This coefficient has a broad minimum at m in a range of 0.8 to 1.5 (Supplement 1, Fig. S1). This range is considered the optimum in terms of measurement time efficiency, at which the equal uncertainty requires $SNR_3(f_B) \approx 1.5 SNR_1$, a modest penalty due to the frequency scan.

Any optical loss from the sample to detectors reduce SNR. For example, the need of an ultrahigh-resolution high-extinction spectrometer [33] can impose substantial optical loss and thus SNR penalty to spontaneous Brillouin spectroscopy. With such practical factors in mind, the comparison in Fig. 2 can serve as a reference for instrumental benchmarking.

2. Experimental results

2.1. System configuration

Figure 3 shows a schematic of the experimental setup we constructed for pulsed Brillouin microscopy. Two separate external-cavity tunable semiconductor diode lasers (Toptica TA Pro for the pump and Toptica DL Pro with BoosTA pro laser amplifier for the probe) were used, each emitting single-mode output at $\lambda = 780$ nm with less than 500 kHz linewidths. The output frequency of the probe laser is locked to the rubidium atom D2 line in a vapor cell. The output frequency of the pump laser is detuned through sweeping the piezo voltage of the laser. The beating signal between the two laser outputs is detected by a fast photodetector (2 GHz bandwidth) and monitored with a rf spectrum analyzer. Both the pump and probe laser beams pass through Pockels electro-optic modulators (EOM, BME KG) and polarization optics to form optical pulses. The pulse duration and repetition rate are tunable and determined by the EOM drive signal from a pulse generator. The shortest pulse width possible is ~ 10 ns. The probe and pump beams enter a sample in the opposite direction and the opposite circular polarizations (same circularity in the lab frame of reference). The outgoing probe beam is separated from the pump beam path by a polarization beam splitter (PBS). The probe beam carrying Brillouin signal is directed to pass through a pair of temperature stabilized narrowband etalons to suppress back-reflections and elastic scattering of the pump beam into the probe beam path. The probe beam is focused onto one port of a dual balanced detector (BD) with a bandwidth of 10 MHz (Newport Model 2107). The other port of BD is supplied by a probe beam tapped from the input beam path with an appropriate fiber-optic delay line. The length matching between the two dual balancing beams is critical for effective RIN suppression. The electrical signal from BD is sampled by a digitizer NI5734 at 120 MS/s and data is directly processed in a field-programmable gate array (FPGA) module (National Instruments, PXIe-7961R).



Fig. 3. Schematic of the microscope system. BE: beam expander, BD: balanced detector, BPF: 780 nm bandpass filter ($\Delta\lambda$ =3 nm), BS: beam splitter, CCD: charged coupled device camera, DBC: dichroic beam combiner, EOM: electro-optic modulator, FPBS: fiber polarization beam splitter, Obj: objective lens (60X NA = 1.1), OP: polarization maintaining optical fiber, PBS: polarization beam splitter, PM: polarization maintaining fiber delay, P1 and P2: photodiodes, $\lambda/2$: half-wave plate, and $\lambda/4$: quarter-wave plate.

In the signal processing, the acquired BD signal undergoes boxcar averaging with an adjustable time window and delay in real-time using FPGA. The same averaging spans across the probe pulse with and without SBS signal and returns a value respectively as signal (n1) and reference (n0) slots. The signal slot is then subtracted from the reference slot and return a difference slot. The interlaced subtraction is immune to slow intensity variations that may occur due to sample scan and mechanical vibrations as well as RIN components below the pump repetition rate. The processed spectral data are sent to a main computer for further data processing including frequency calibration and curve fittings.

2.2. Characterization of SNR and uncertainties

Figure 4(a) shows a typical Brillouin spectrum obtained from distilled water with a pulse width of 100 ns at a period of 2 µs for the pump and 1 µs for the probe. Therefore, $\kappa = 0.05$. The optical powers at the sample are: $P_p = 1$ W, $\langle P_p \rangle = 50$ mW, $P_r = 50$ mW, $\langle P_r \rangle = 5$ mW (twice the duty cycle of the pump). The peak probe power at the detector was 20 mW, slightly below the saturation power of the detector. To acquire the entire Brillouin spectra, the probe frequency was linearly swept over a range of 2 GHz in 20 ms. For each frequency sweep, 10,000 boxcar data (n1-n0) are acquired with a boxcar integration window size of 280 ns. For real-time display and post-analysis, the data are typically reduced to 100 spectral points. Therefore, the effective integration time of each spectral point is T = 200 µs. Each gain spectrum is curve-fitted with Lorentzian function to retrieve the amplitude, frequency shift, linewidth Γ . The noise σ within



Fig. 4. Experimental data obtained from water. (a) Brillouin gain spectrum obtained with 100 ns pulses. The measured peak SNR at 5.05 GHz is 1280, and the linewidth is 316 MHz. (b) SNR vs. pump peak power at $P_r = 50$ mW, (c) SNR vs. probe peak power at $P_p = 1$ W. SNR over 1000 is measured with $\langle P_p \rangle = 45$ mW. (d) Brillouin frequency shift uncertainty σ_{fB} vs. probe peak power at $P_p = 1$ W. (e) SNR vs. pulse width at $P_p = 1$ W and $P_r = 50$ mW, (f) SNR vs. pulse width τ at $\langle P_p \rangle = 10$ mW and $P_r = 50$ mW. With $\kappa = 0.01$, SNR over 1000 is measured with $\langle P_{tot} \rangle = 10$ mW. Error bars: standard deviations over 100 measurements. The data integration time is 200 µs in (b-e) and 2 ms (f). Circles, experimental data. Red curves, theory (polynomials).

the spectrum is computed from the standard deviation of the data outside the Brillouin gain profile. SNR is determined as the ratio of the amplitude squared to the noise squared.

We acquired Brillouin spectra of distilled water over 100 repeated frequency sweeps and analyzed statistical variations. The gain coefficient b is determined to be $\sim 1 \times 10^{-4}$ m²/W from the spectra. Careful optical alignment is critical in obtaining the theoretical gain coefficient. The SNR increases quadratically with the pump peak power, as expected, in Fig. 4(b). SNR increases linearly with the probe power as in Fig. 4(c). This shows evidence that the detection scheme is shot noise limited. A detailed SNR analysis specific to the interlaced boxcar signal processing is described in Supplemental document. The linear increase of SNR leads to the reciprocal decrease of Brillouin frequency uncertainty, as shown in Fig. 4(d). In Fig. 4(e), SNR increases linearly with the pulse width of the pump and probe while their peak powers are held constant. This is consistent with Eq. (4) when both the total average power and duty cycle are proportional to the pulse width. To verify the dependence on the pulse duty cycle, we vary the pulse width for the same repetition rates (0.5 MHz for pump and 1 MHz for probe) at constant average pump power and constant peak probe power. As expected, SNR increases with the reciprocal of duty cycle, as shown in Fig. 4(f). Reducing the duty cycle to below 0.01 is expected to increase SNR even further until the pulse duration is comparable to the lifetime of acoustic waves, which is on the order ~ 1 ns. However, due to instrumentation constraints, this limit was not tested in our study. With the measured Brillouin linewidth of 318 MHz for water, the scan range of 2 GHz makes $m \approx 6.3$, which is far more than necessary for time-efficient spectroscopy. The measured uncertainties were the same when we used only the spectral data between 4.7 and 5.3 GHz for curve fitting. This corresponds to a spectral integration time of 6 ms.

3. Cellular imaging and time-lapse fluctuations

Brillouin imaging is demonstrated with HeLa cancer cells. The prepared cells attached on the glass of a glass bottom dish filled with buffer solution. The laser focal spot size is about 0.7 μ m, and the pixel interval is 0.25 μ m. The acquisition time per pixel is 20 ms. The same 2 GHz scan range was used. Figure 5 show the cellular maps of Brillouin frequency shift, amplitude, and linewidth. Nucleoli are clearly identified by higher frequency shifts. Linewidths tend to be higher in the cytoplasm than in the nucleus. The Brillouin linewidth is related to the reciprocal of the finite lifetime of Brillouin-generated acoustic waves due to their absorption and scattering in the medium. An inverse correlation between the Brillouin gain and linewidth is apparent. The total number of Brillouin scattered photons over the full spectrum is independent of the Brillouin linewidth in both stimulated and spontaneous scattering.

Figure 6 show the time-lapse traces measured with an integration time of 20 ms. The nucleolus has the largest uncertainty in Brillouin frequency shift. From the time-lapse data, we measure $f_B = 5.11 \pm 0.0079$ GHz and $\Gamma = 0.38 \pm 0.031$ GHz in the cell medium. In the nucleolus, we measure $f_B = 5.51 \pm 0.03$ GHz and $\Gamma = 1.01 \pm 0.118$ GHz. From the Brillouin data, we can determine the complex longitudinal modulus, M = M' + iM''. We derive

$$M' = \rho v^2 \frac{1 - \gamma^2}{\left(1 + \gamma^2\right)^2} \tag{6}$$

$$M'' = \rho v^2 \frac{2\gamma}{\left(1 + \gamma^2\right)^2}$$
(7)

where $v = \frac{\lambda}{2n} f_B$ and $\gamma \equiv \frac{\Gamma}{f_B}$. When $\gamma \ll 1$, $M' \approx \rho v^2 (1 - 3\gamma^2)$ and $M'' \approx 2\rho v^2 \gamma$ (Supplement 1). For the cell medium, we obtain M' = 2.36 GPa and M'' = 0.96 GPa. For the nucleolus, (using $\rho/n^2 = 0.565$ g/cm³, the same as water) we obtain M' = 2.20 GPa and M'' = 0.33 GPa. The histograms show normal distributions. With an increased integration time of 200 ms, σ_f is reduced to 3.1 MHz and 7.8 MHz and, with 2 s integration, to 0.66 MHz and 3.3 MHz in the



Fig. 5. Brillouin images of live cells in vitro. (a) Brillouin frequency shift, gain, and linewidth image of a HeLa cancer cell, accompanied with bright-field images (gray) and confocal fluorescent images (indigo: nucleus, red: membrane). Number of pixels: 100×240 (H x V) in (a) and 200×100 in (b).



Fig. 6. Time-lapse fluctuations of Brillouin parameters at fixed locations in a sample. The spectral acquisition time is 20 ms. (a) Brillouin frequency shift, (b) Linewidth and amplitude in the extracellular culture medium, (c) Linewidth and amplitude in the nucleolus, and (d) histogram of Brillouin frequency in the nucleolus with a Gaussian fit.

medium and nucleolus, respectively. In this dataset the fluctuations are governed by SNR and apparently not by other physical or biological changes. Further investigation of intracellular dynamics is beyond the scope of this paper.

4. Conclusion

Using the pulse scheme for SBS microscopy brings marked precision over exposed laser power benefits as compared with spontaneous Brillouin and cw SBS schemes. The interlaced boxcar method was effective to retrieve the SBS signal with shot noise-limited SNR. No signs of photodamage were observed in the cells during the entire experiment. In a long-term operation, the pulse width is currently limited to 100 ns due to excessive heating in electronics, resulting in a duty cycle of 0.05. The optical power efficiency is expected to be improved further by reducing the pulse width to 10 ns (duty cycle of 0.005) and raising the peak power of pump on sample, while setting the average power to a desired level. Compared to spontaneous Brillouin microscopy, SBS microscopy requires optical access to a sample from two opposite sides. However, this disadvantage can be managed in many *in vitro* and on-chip settings, as well as for thin tissue slices. Our results show that pulsed SBS microscopy can provide better SNR performance for interrogating such biological samples.

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Data availability. Data underlying the results presented in this paper are not publicly available at this time but may be obtained from the authors upon reasonable request.

Supplemental document. See Supplement 1 for supporting content.

References

- G. Scarcelli and S. H. Yun, "Confocal Brillouin microscopy for three-dimensional mechanical imaging," Nat. Photonics 2(1), 39–43 (2008).
- J. Margueritat, A. Virgone-Carlotta, S. Monnier, H. Delanoë-Ayari, H. C. Mertani, A. Berthelot, Q. Martinet, X. Dagany, C. Rivière, J.-P. Rieu, and T. Dehoux, "High-Frequency Mechanical Properties of Tumors Measured by Brillouin Light Scattering," Phys. Rev. Lett. 122(1), 018101 (2019).
- J. Rix, O. Uckermann, K. Kirsche, G. Schackert, E. Koch, M. Kirsch, and R. Galli, "Correlation of biomechanics and cancer cell phenotype by combined Brillouin and Raman spectroscopy of U87-MG glioblastoma cells," J. R. Soc. Interface. 19(192), 20220209 (2022).
- G. Scarcelli, W. J. Polacheck, H. T. Nia, K. Patel, A. J. Grodzinsky, R. D. Kamm, and S. H. Yun, "Noncontact three-dimensional mapping of intracellular hydromechanical properties by Brillouin microscopy," Nat. Methods 12(12), 1132–1134 (2015).
- G. Antonacci and S. Braakman, "Biomechanics of subcellular structures by non-invasive Brillouin microscopy," Sci. Rep. 6(1), 37217 (2016).
- R. Schlüßler, S. Möllmert, S. Abuhattum, G. Cojoc, P. Müller, K. Kim, C. Möckel, C. Zimmermann, J. Czarske, and J. Guck, "Mechanical Mapping of Spinal Cord Growth and Repair in Living Zebrafish Larvae by Brillouin Imaging," Biophys. J. 115(5), 911–923 (2018).
- A. M. Eltony, P. Shao, and S.-H. Yun, "Measuring mechanical anisotropy of the cornea with Brillouin microscopy," Nat. Commun. 13(1), 1354 (2022).
- G. Scarcelli, R. Pineda, and S. H. Yun, "Brillouin Optical Microscopy for Corneal Biomechanics," Invest. Ophthalmol. Vis. Sci. 53(1), 185 (2012).
- M. Bailey, M. Alunni-Cardinali, N. Correa, S. Caponi, T. Holsgrove, H. Barr, N. Stone, C. P. Winlove, D. Fioretto, and F. Palombo, "Viscoelastic properties of biopolymer hydrogels determined by Brillouin spectroscopy: A probe of tissue micromechanics," Sci. Adv. 6(44), eabc1937 (2020).
- B. Krug, N. Koukourakis, and J. W. Czarske, "Impulsive stimulated Brillouin microscopy for non-contact, fast mechanical investigations of hydrogels," Opt. Express 27(19), 26910 (2019).
- I. I. L. Fabelinskiĭ, "Spectra of molecular light scattering and some of their applications," Phys.-Usp. 37(9), 821–858 (1994).
- 12. G. Benedek and T. Greytak, "Brillouin scattering in liquids," Proc. IEEE 53(10), 1623-1629 (1965).
- R. Y. Chiao, C. H. Townes, and B. P. Stoicheff, "Stimulated Brillouin Scattering and Coherent Generation of Intense Hypersonic Waves," Phys. Rev. Lett. 12(21), 592–595 (1964).
- C. W. Ballmann, J. V. Thompson, A. J. Traverso, Z. Meng, M. O. Scully, and V. V. Yakovlev, "Stimulated Brillouin Scattering Microscopic Imaging," Sci. Rep. 5(1), 18139 (2015).
- T. Li, F. Li, X. Liu, V. V. Yakovlev, and G. S. Agarwal, "Quantum-enhanced stimulated Brillouin scattering spectroscopy and imaging," Optica 9(8), 959 (2022).

- R. W. Boyd, K. Rzaąewski, and P. Narum, "Noise initiation of stimulated Brillouin scattering," Phys. Rev. A 42(9), 5514–5521 (1990).
- I. Remer, R. Shaashoua, N. Shemesh, A. Ben-Zvi, and A. Bilenca, "High-sensitivity and high-specificity biomechanical imaging by stimulated Brillouin scattering microscopy," Nat. Methods 17(9), 913–916 (2020).
- R. A. Hoebe, C. H. Van Oven, T. W. J. Gadella, P. B. Dhonukshe, C. J. F. Van Noorden, and E. M. M. Manders, "Controlled light-exposure microscopy reduces photobleaching and phototoxicity in fluorescence live-cell imaging," Nat. Biotechnol. 25(2), 249–253 (2007).
- J. Icha, M. Weber, J. C. Waters, and C. Norden, "Phototoxicity in live fluorescence microscopy, and how to avoid it," BioEssays 39(8), 1700003 (2017).
- T. Horiguchi, K. Shimizu, T. Kurashima, M. Tateda, and Y. Koyamada, "Development of a distributed sensing technique using Brillouin scattering," J. Lightwave Technol. 13(7), 1296–1302 (1995).
- D. M. Chow, Z. Yang, M. A. Soto, and L. Thévenaz, "Distributed forward Brillouin sensor based on local light phase recovery," Nat. Commun. 9(1), 2990 (2018).
- M. Santagiustina, S. Chin, N. Primerov, L. Ursini, and L. Thévenaz, "All-optical signal processing using dynamic Brillouin gratings," Sci. Rep. 3(1), 1594 (2013).
- M. Merklein, B. Stiller, and B. J. Eggleton, "Brillouin-based light storage and delay techniques," J. Opt. 20(8), 083003 (2018).
- 24. L. Thévenaz, "Slow and fast light in optical fibres," Nat. Photonics 2(8), 474-481 (2008).
- N. Chauhan, A. Isichenko, K. Liu, J. Wang, Q. Zhao, R. O. Behunin, P. T. Rakich, A. M. Jayich, C. Fertig, C. W. Hoyt, and D. J. Blumenthal, "Visible light photonic integrated Brillouin laser," Nat. Commun. 12(1), 4685 (2021).
- N. T. Otterstrom, R. O. Behunin, E. A. Kittlaus, Z. Wang, and P. T. Rakich, "A silicon Brillouin laser," Science 360(6393), 1113–1116 (2018).
- B. J. Eggleton, C. G. Poulton, P. T. Rakich, M. J. Steel, and G. Bahl, "Brillouin integrated photonics," Nat. Photonics 13(10), 664–677 (2019).
- 28. P. Shao, A. M. Eltony, T. G. Seiler, B. Tavakol, R. Pineda, T. Koller, T. Seiler, and S.-H. Yun, "Spatially-resolved Brillouin spectroscopy reveals biomechanical abnormalities in mild to advanced keratoconus in vivo," Sci. Rep. 9(1), 7467 (2019).
- 29. S. Minin and F. Kamalabadi, "Uncertainties in extracted parameters of a Gaussian emission line profile with continuum background," Appl. Opt. 48(36), 6913 (2009).
- M. A. Soto and L. Thévenaz, "Modeling and evaluating the performance of Brillouin distributed optical fiber sensors," Opt. Express 21(25), 31347 (2013).
- R. E. Thompson, D. R. Larson, and W. W. Webb, "Precise Nanometer Localization Analysis for Individual Fluorescent Probes," Biophys. J. 82(5), 2775–2783 (2002).
- G. Zanini and G. Scarcelli, "Localization-assisted stimulated Brillouin scattering spectroscopy," APL Photonics 7(5), 056101 (2022).
- G. Scarcelli and S. H. Yun, "Multistage VIPA etalons for high-extinction parallel Brillouin spectroscopy," Opt. Express 19(11), 10913 (2011).